Viral binding at 4°C was also competed by the addition of free heparin at the indicated concentrations shown in Figure 18C [Panel C] (confocal images shown from three merged layers). Endocytosis of FITC-labeled transferrin and Cy3-labeled rAAV was observed in Hela cells (Figure 18D [Panel D]). Hela cells were infected with Cy3 rAAV in the presence of FITC-labeled transferrin for 90 minutes at 4°C followed by washing. Cells were then placed at 37°C for 30 minutes prior to fixation and analysis by confocal microscopy. Images in Figure 18D [Panel D} were a single 0.5 μm cross section for Cy3, FITC, and combined channels (merged). Results demonstrate colocalization of rAAV and transferrin in the majority of endocytic vesicles.

In the Claims

Please substitute the claim set in the appendix entitled "Clean Version of Pending Claims" for the previously pending claim set. The specific amendments to individual claims are detailed in the following marked-up set of claims.

Please amend the claims as follows:

1. (Amended) A method to identify an agent that alters adeno-associated virus (AAV) transduction of a mammalian cell, comprising:
a) contacting the mammalian cell with [the] an agent and virus; and
b) detecting or determining whether [virus transduction is altered] the agent alters viral transduction, wherein the agent alters transduction after viral binding to receptors and before synthesis to an expressible form of the viral genome.

6. (Amended) The method of claim 1 wherein the agent enhances endosomal processing [is enhanced].

12. (Amended) The method of claim 10 wherein the recombinant virus comprises a marker gene that is detectable or selectable [or a selectable gene].

Please add the following new claims:

Docket No. 00875.032US1 Client Reference Number N9-67
WD #476020.WPD

CLEAN VERSION OF PENDING CLAIMS

COMPOUNDS AND METHODS TO ENHANCE rAAV TRANSDUCTION
Applicant: John F. Engelhardt et al.
Serial No.: 09/689,136

Claims 1-12, 29-36 and 83-86, as of November 26, 2002, date of response to first Office Action. (NOTE: Non-elected claims 13-28 and 37-82 have been withdrawn from consideration.)

1. A method to identify an agent that alters adeno-associated virus (AAV) transduction of a mammalian cell, comprising:
   a) contacting the mammalian cell with an agent and virus; and
   b) detecting or determining whether the agent alters viral transduction, wherein the agent alters transduction after viral binding to receptors and before synthesis to an expressible form of the viral genome.

2. The method of claim 1 wherein the cell is a mammalian lung cell.

3. The method of claim 1 wherein the cell is a mammalian liver cell.

4. The method of claim 1 wherein the cell is a human cell, canine cell, murine cell, rat cell or rabbit cell.

5. The method of claim 1 wherein the transduction is enhanced.

6. The method of claim 1 wherein the agent enhances endosomal processing.

7. The method of claim 1 wherein the agent is an endosomal protease inhibitor.

8. The method of claim 7 wherein the agent is a cysteine protease inhibitor.

9. The method of claim 1 wherein the agent is a peptide or analog thereof.

10. The method of claim 1 wherein the virus is recombinant adeno-associated virus.

11. The method of claim 10 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.

C11

12. The method of claim 10 wherein the recombinant virus comprises a marker gene that is detectable or selectable.

29. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (I): R_1-A-$(B)_n$-C wherein R_1 is an N-terminal amino acid blocking group; each A and B is independently an amino acid; C is an amino acid wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and n is 0, 1, 2, or 3; or a pharmaceutically acceptable salt thereof.

30. The method of claim 29 wherein R_1 is (C_1-C_{10})alkanoyl.

31. The method of claim 29 wherein R_1 is acetyl or benzyloxycarbonyl.

32. The method of claim 29 wherein each A and B is independently alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine.

33. The method of claim 29 wherein each A and B is isoleucine.

34. The method of claim 29 wherein C is alanine, arginine, glycine, isoleucine, leucine, valine, nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced

by a formyl (CHO) group.

35. The method of claim 29 wherein C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group.

36. The method of claim 29 wherein R_1 is (C_1-C_{10})alkanoyl or benzyloxycarbonyl; A and B are each isoleucine; C is nor-leucine or nor-valine, wherein the terminal carboxy group has been replaced by a formyl (CHO) group; and N is 1.

83. The method of claim 1, 13, 14, 15, 15 or 17 further comprising administering a second agent that enhances the activity of the agent.

84. The method of claim 83 wherein the second agent is EGTA.

85. (New) The method of claim 1 wherein the agent is an ubiquitin ligase inhibitor.

86. (New) The method of claim 1 wherein the agent alters endosomal processing.

WITHDRAWN:

13. A method to alter adeno-associated virus transduction of a mammalian lung cell, comprising: contacting the mammalian lung cell with an amount of an agent and an amount of virus effective to alter virus transduction.

14. A method to alter adeno-associated virus transduction of a mammalian liver cell, comprising: contacting the mammalian liver cell with an amount of an agent and an

amount of virus effective to alter virus transduction.

15. A method to alter the expression of a transgene in a mammalian lung cell, comprising: contacting the mammalian lung cell with an amount of an agent and an amount of recombinant adeno-associated virus comprising the transgene so as to alter expression of the transgene.

16. A method to alter the expression of a transgene in a mammalian liver cell, comprising: contacting the mammalian liver cell with an amount of an agent and an amount of recombinant adeno-associated virus comprising the transgene so as to alter expression of the transgene.

17. A method comprising: contacting a mammal subjected to gene therapy with recombinant adeno-associated virus comprising a transgene with an amount of an agent effective to alter expression of the transgene in the cells of the mammal.

18. The method of claim 13, 14, 15, 16, or 17 wherein endosomal processing of the virus is altered.

19. The method of claim 13 or 14 wherein the virus is recombinant adeno-associated virus.

20. The method of claim 19 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.

21. The method of claim 13 or 14 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.

22. The method of claim 15, 16, or 17 wherein the recombinant virus encodes a therapeutic peptide or polypeptide.

23. The method of claim 13, 14, 15, 16 or 17 wherein the cell is contacted with the agent before the cell is contacted with the virus.

24. The method of claim 13, 14, 15, 16 or 17 wherein the cell is contacted with the virus before the cell is contacted with the agent.

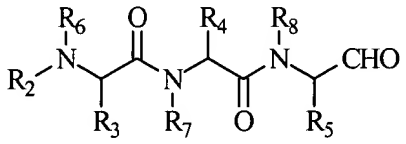
25. The method of claim 13, 14, 15, 16 or 17 wherein virus transduction is enhanced.

26. The method of claim 15, 16 or 17 wherein transgene expression is enhanced.

27. The method of claim 17 wherein expression is altered in lung cells.

28. The method of claim 17 wherein expression is altered in liver cells.

37. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (II):



(II)

wherein

R_2 is an N-terminal amino acid blocking group;

R_3, R_4, and R_5 are each independently hydrogen, (C_1-C_{10})alkyl, aryl or aryl(C_1-C_{10})alkyl; and

R_6, R_7, and R_8 are each independently hydrogen, $(C_1$-$C_{10})$alkyl, aryl or aryl$(C_1$-$C_{10})$alkyl; or a pharmaceutically acceptable salt thereof.

38. The method of claim 37 wherein R_2 is $(C_1$-$C_{10})$alkanoyl.

39. The method of claim 37 wherein R_2 is acetyl or benzyloxycarbonyl.

40. The method of claim 37 wherein R_3 is hydrogen or $(C_1$-$C_{10})$alkyl.

41. The method of claim 37 wherein R_3 is 2-methylpropyl.

42. The method of claim 37 wherein R_4 is hydrogen or $(C_1$-$C_{10})$alkyl.

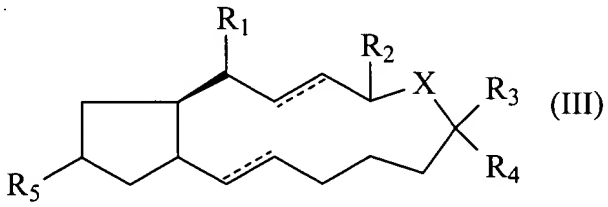
43. The method of claim 37 wherein R_4 is 2-methylpropyl.

44. The method of claim 37 wherein R_5 is hydrogen or $(C_1$-$C_{10})$alkyl.

45. The method of claim 37 wherein R_5 is butyl or propyl.

46. The method of claim 37 wherein R_2 is acetyl or benzyloxycarbonyl; R_3 and R_4 are each 2-methylpropyl; R_5 is butyl or propyl; and R_6, R_7, and R_8 are each independently hydrogen.

47. The method of claim 1, 13, 14, 15, 16 or 17 wherein the agent is a compound of formula (III):



(III)

wherein

R_1 is H, halogen, $(C_1$-$C_{10})$alkyl, $(C_1$-$C_{10})$alkenyl, $(C_1$-$C_{10})$alkynyl, $(C_1$-$C_{10})$alkoxy, $(C_1$-$C_{10})$alkanoyl, (=O), (=S), OH, SR, CN, NO_2, trifluoromethyl or $(C_1$-$C_{10})$alkoxy, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN, NO_2, trifluoromethyl, NRR or SR, wherein each R is independently H or $(C_1$-$C_{10})$alkyl;

R_2 is (=O) or (=S);

R_3 is H, $(C_1$-$C_{10})$alkyl, $(C_1$-$C_{10})$alkenyl, $(C_1$-$C_{10})$alkynyl, $(C_1$-$C_{10})$alkoxy or $(C_3$-$C_8)$cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO_2, trifluoromethyl, SR, or NRR, wherein each R is independently H or $(C_1$-$C_{10})$alkyl;

R_4 is H, $(C_1$-$C_{10})$alkyl, $(C_1$-$C_{10})$alkenyl, $(C_1$-$C_{10})$alkynyl, $(C_1$-$C_{10})$alkoxy or $(C_3$-$C_8)$cycloalkyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or cycloalkyl may optionally be substituted with one or more halogen, OH, CN, NO_2, trifluoromethyl, SR, or NRR, wherein each R is independently H or $(C_1$-$C_{10})$alkyl;

R_5 is H, halogen, $(C_1$-$C_{10})$alkyl, $(C_1$-$C_{10})$alkenyl, $(C_1$-$C_{10})$alkynyl, $(C_1$-$C_{10})$alkoxy, $(C_1$-$C_{10})$alkanoyl, (=O), (=S), OH, SR, CN, NO_2 or trifluoromethyl, wherein any alkyl, alkenyl, alkynyl, alkoxy or alkanoyl may optionally be substituted with one or more halogen, OH, SH, CN, NO_2, trifluoromethyl, NRR or SR, wherein each R is independently H or $(C_1$-$C_{10})$alkyl; and

X is O, S or NR wherein R is H or $(C_1$-$C_{10})$alkyl, or a pharmaceutically acceptable salt thereof.

48. The method of claim 47 wherein R_1 is halogen, CN, NO_2, trifluoromethyl or OH.

49. The method of claim 47 wherein R_1 is OH.

50. The method of claim 47 wherein R_2 is (=O).

51. The method of claim 47 wherein R_3 is H or $(C_1$-$C_{10})$alkyl.

52. The method of claim 47 wherein R_3 is methyl.

53. The method of claim 47 wherein R_4 is H or $(C_1$-$C_{10})$alkyl.

54. The method of claim 47 wherein R_4 is H.

55. The method of claim 47 wherein R_5 is halogen, CN, NO_2, trifluoromethyl or OH.

56. The method of claim 47 wherein R_5 is OH.

57. The method of claim 47 wherein X is O or S.

58. The method of claim 47 wherein X is O.

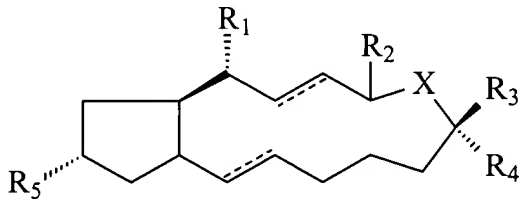
59. The method of claim 47 wherein both ----- are a single bond.

60. The method of claim 47 wherein one ----- is a double bond.

61. The method of claim 47 wherein both ----- are a double bond.

62. The method of claim 45 wherein R_1 is OH, R_2 is (=O), R_3 is methyl, R_4 is H, R_5 is OH, X is O, and both ----- are a double bond.

63. (Amended) The method of claim 47 wherein the compound is a compound of formula (III):



III

64. The method of claim 63 wherein R_1 is halogen, CN, NO_2, trifluoromethyl or OH.

65. The method of claim 63 wherein R_1 is OH.

66. The method of claim 63 wherein R_2 is (=O).

67. The method of claim 63 wherein R_3 is H or (C_1-C_{10})alkyl.

68. The method of claim 63 wherein R_3 is methyl.

69. The method of claim 63 wherein R_4 is H or (C_1-C_{10})alkyl.

70. The method of claim 63 wherein R_4 is H.

71. The method of claim 63 wherein R_5 is halogen, CN, NO_2, trifluoromethyl or OH.

72. The method of claim 63 wherein R_5 is OH.

73. The method of claim 63 wherein X is O or S.

74. The method of claim 63 wherein X is O.

75. The method of claim 63 wherein both ----- are a single bond.

76. The method of claim 63 wherein one ----- is a double bond.

77. The method of claim 63 wherein both ----- are a double bond.

78. The method of claim 63 wherein R_1 is OH, R_2 is (=O), R_3 is methyl, R_4 is H, R_5 is OH, X is O, and both ----- are a double bond.

79. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent inhibits the activation of ubiquitin, the transfer of ubiquitin to the ubiquitin carrier protein, ubiquitin ligase, or a combination thereof.

80. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent inhibits ubiquitin ligase.

81. The method of claim 1, 13, 14, 15, 15 or 17 wherein the agent is a compound of formula (IV):

$$R\text{—}A\text{—}A_1\text{—}R_1$$

wherein R is hydrogen, an amino acid, or a peptide, wherein the N-terminus amino acid can optionally be protected at the amino group with acetyl, acyl, trifluoroacetyl, or benzyloxycarbonyl; A is an amino acid or a direct bond; A_1 is an amino acid; and R_1 is hydroxy or an amino acid, wherein the C-terminus amino acid can optionally be protected at the carboxy group with $(C_1\text{-}C_6)$alkyl, phenyl, benzyl ester or amide (e.g., $C(=O)NR_2$, wherein each R is independently hydrogen or $(C_1\text{-}C_6)$alkyl);
or a pharmaceutically acceptable salt thereof.

82. The method of claim 81 wherein the agent is H-Leu-Ala-OH, H-His-Ala-OH, or a combination thereof.